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Research Article

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In Vitro Regeneration of *Muscari racemosum* Mill. Using Twin Bulb Scales, Primary Bulbs, and Leaf Bases

Abstract
Turkey is an important center of diversity for many plants species including bulbs, rhizomes, tubers, and other plants of high agricultural and horticultural importance. These species have a special importance as ornamental plants. However, due to urbanization and related factors, many of them are under threat. One of these species is the endemic *Muscari racemosum* Mill. The current study aimed to develop an efficient *in vitro* commercial bulblet propagation procedure using different explants. Twin-scale bulb explants were regenerated on MS medium having several doses of Kinetin+NAA (1-Naphthaleneacetic acid). The best regeneration was exhibited on 4.65 µM Kinetin+5.37 µM NAA at the end of 10 weeks with induction of 4.08 bulblets/explant with a mean diameter of 0.31 cm. The primary bulblets were cultured on MS medium having 18.60 µM Kinetin+5.37 µM NAA. About a 2.5-fold increase in the diameter of the bulbs (0.76 cm) was exhibited on the regenerated bulblets. The bulblets were regenerated on leaf bases taken from MS medium having several doses of BAP (6-Benzylaminopurine) + NAA. The regenerated bulbs were rooted on MS medium having 4.90 µM IBA (Indole-3-butyric acid) followed by their transference to a greenhouse for acclimatization. This study provided important information on commercial clonal propagation of *M. racemosum* and the importance of explants and growth regulators in plant regeneration.

INTRODUCTION

Turkey lies on the intersection of three floristic regions and has a rich plant diversity with >1056 taxa (bulbs, rhizomes, and tubers), of which 424 species are endemic (Altuntaş, 2020; Yıldırım, 2020). One of the remarkable monocotyledonous bulbous genera among these geophytes is Muscari Mill. with high commercial value globally (Kocak et al., 2019). The genus Muscari was previously included in the Liliaceae family. It was later revised and included in the Hyacinthaceae family. This classification was re-revised, now the genus is included in the Asparagaceae family (Eroğlu, 2020). There are over 50 reported species in genus Muscari in all of Europe, the Mediterranean region, and Southwest Asia (Chittenden, 1956; Van Scheepen, 1991; Jafari and Maassoumi, 2011; Govaerts, 2019; Yıldırım, 2020). After the latest revision of the genus, Muscari in Turkey, the latest checklist (Eker, 2012) has listed 49 new species (Yildirimli, 2010; Eker, 2012; Eker, et al., 2019; Kayiran et al., 2019; Eker, 2019a, b; Eker, et al., 2020a, b; TÜBİVES, 2021; IPNI, 2021; WCSP, 2021). Rapid urbanization (forest fires, construction of houses, unconscious collections of flowering plants by the hobbyists, increased tourism-based activities, construction and widening of roads, developing mines, and residual accumulation of poisonous wastes from factories) along with increased intrusions on forest lands for agriculture and overgrazing of plant-soil cover for animal feed, increased use of pesticides, herbicides, and chemical fertilizers, etc. have negative contributions to the development of wild flora. The species in the genus Muscari are crucial geophytes with commercial importance in the ornamental and medicinal plants sector (Şengün and Yücel, 2018; Meydan, 2019). Therefore, the researchers have focused on their cytogenetic (Demirci Kayıran and Özhatay, 2017), molecular (Al-Sammarraie, 2020), morphological, and anatomical (Gürsoy and Şık, 2010; Gürsoy, 2016; İlçim et al., 2020) features to understand their characteristics and produce them commercially. Establishing large protected areas and rapid multiplication of these plants using tissue culture studies for conservation are of special importance. Various *in vitro* multiplication and agronomic techniques are desired to conserve and multiply them (Ozel, 2008; da Silva and Dobránszki, 2016; Kocak et al., 2019). Both explant source and plant growth regulators used are very critical factors in plant regeneration and multiplication. Explant sources such as embryonic calli, 2-5 bulb scales, basal layer of the bulbs, leaves, stems, and immature zygotic embryos have been used to stimulate regeneration in several species of genus Muscari (Ozel et al., 2007; Ozel, 2008; Ozel et al., 2009; Vaziri et al., 2009; Uranbey, 2010a, b; Uranbey et al., 2010; Nasırcılar et al., 2011; Uzun et al., 2014; Ozel et al., 2015; Yücesan et al., 2014; Ozel et al., 2016; Özdemir et al., 2017 and Fida, 2020). However, there is a need to optimize the ratio of auxin, cytokinin, and other plant growth regulators for regeneration. Therefore, the researchers have generally preferred 6-Benzylaminopurine (BAP), Kinetin, thidiazuron (TDZ), Zeatin, Picloram, 2,4-Dichlorophenoxyacetic acid (2,4-D), Naphthaleneacetic acid (NAA), Indole-3-butyric acid (IBA), and 3-Indoleacetic acid (IAA), (Ozel, 2008; Uranbey, 2010a; Nasırcılar et al., 2011; Vaziri et al., 2014; Özdemir et al. 2017; Fida, 2020). One of the most important species in the genus Muscari is *Muscari racemosum* (syn. *Muscari muscarimi*) (Eker, 2012; IPNI, 2021; WCSP, 2021). It is an endemic species in Antalya Section (6a) (Eker, 2012). *M. racemosum* bulbs are 2-4 cm in diameter. The flowers of this species bloom in May and June, are musky, violet in the early periods, dirty grayish-white or greenish at the late stages of flowering, and brown in color before dying. The unproductive flowers are small and violet in color (TUBİVES, 2021). There are only a couple of *in vitro* studies of *Muscari racemosum* [syn. *M. muscarimi*] (Kromer,

1988; Uzun et al., 2014; Ozel et al., 2015). The target of the current study was to multiply Muscari wild bulb scales, *in vitro* grown bulbs, and leaf bases as explants. Therefore, bulb scales and *in vitro* grown bulbs were allowed to regenerate on a medium having several doses of Kinetin + NAA. However, leaf bases were regenerated on several doses of BAP + NAA.

MATERIAL and METHODS

Source of *M. racemosum* and Surface Sterilization

The *M. racemosum* bulbs were gathered from the Department of Field Crops, Ankara University, Turkey. They were washed in commercial detergent (Haci Sakir Turkey) for about 40 min under running tap water followed by their drying. The clean and dried bulbs with a diameter of 1.25-1.50 cm were stored at room temperature (24±1°C) in a cool ventilated shed for eight weeks avoiding fungus development during storage. Thereafter, the healthy bulbs without any bruise or visual signs of contamination were selected for taking explants. The outer scales and roots of each bulb were taken away using sterilized scalpel blades before subjecting them to surface sterilization with 80% commercial bleach (Ace, Turkey, having 5% NaOCl) for 20 min. Tween 20 (1/100 mL v/v) was added to the solution as a surfactant. Then the sterilized bulbs were cleaned by rinsing and agitating using sterilized bidistilled water (5 × 3 minutes).

Isolation of Explant, Regeneration, and Rooting

Twin bulbs scale as explant

Each of the sterilized bulbs was vertically sliced into 4 followed by careful separation of two scales (explants) joined by a thin connection. Internal narrow bulb scales were discarded. All explants were micro propagated on MS basal medium having 12 several combinations of 4.65, 9.30, and 18.60 μM Kinetin and 2.685, 5.37, and 10.74 μM NAA to induce regeneration on the explants.

Primary bulblets as explants

The induced primary bulblets were isolated from the mother explants at the end of 10 weeks and subculture for 20 weeks to increase their diameter and induce secondary bulblet formation.Rooting of all regenerated bulbs in these experiments was carried out using 1× MS medium having 4.90 μM IBA.

Leaf bases as explant

The 1 cm long leaf bases from leaf blades induced on the primary bulbs obtained under *in vitro* conditions as previously reported (Ozel et al., 2015) were used as explant by sub-culturing them on MS medium having 4.44, 8.88, and 17.76 μM BAP+ 2.685, 5.37, and 10.74 μM NAA (12 combinations) for regeneration. The induced bulbs with the largest diameter were rooted on MS medium having 4.90 μM IBA.

Culture media, rooting and acclimatization

The pH of all cultures in the MS medium was adjusted to 5.7 using or 1N HCl or 1N NaOH. These cultures were autoclaved for 21 min at 121°C, 104 kPa pressure. These explants were subjected to incubation at 24±1°C 16 h light (35 μmol m^{-2} s^{-1}) day length using Philips-day light lamps TLD 36 W/54, Hungary. Robust well-developed rooted bulbs with green leaves were acclimatized. Care was taken to take away the agar from the bulblets before transference to clay pots having 0.75 liters locally prepared leaf-based peat moss. The peat moss had a pH of 6.0 with EC (electrical conductivity) of 0.1 dS m^{-1} and 67.5% (v/w) porosity. The peat moss allowed water absorption with a bulk density of 0.1 mg m^{-3}. These experimental pots were left at 25 ± 2°C under a 16 h light (35 μmol m^{-2} s^{-1}) day length in the growth chamber. The growth chamber had 80% RH (relative humidity), and the experimental pots were transparent polythene sheet covered to maintain relative humidity.

After the experimental plants began to show signs of development and growth, the polythene covers were pierced for enabling air movement and adjusting for easy acclimatization of the plants to outside environmental conditions. Every experimental pot was watered (60 mL) daily throughout the first week. Thereafter, watering was carried out after every 4 days and continued for 8 weeks. The hardened plants showed visible signs of development and growth. These plants were carefully uprooted without damaging their root structure to find morphological developments on bulb size and the number of roots.

Statistical analysis

All experimental data were analyzed by comparing means using one-way ANOVA (SPSS Ver. 26) (Faraway, 2002). The means showing significant differences among themselves were subjected to post-hoc tests (Tukey's b test) by comparison made at 0.05 and 0.01 levels of significance. Each treatment made using 60 double scale or leaf base explants was divided equally into 15 replications having 4 explants per replicate. The experiments were repeated twice.

RESULT and DISCUSSION

The results of this important study are described below.

Twin Bulb Scales as Explants

The numbers and diameters of the bulbs emerging from the twin bulb scales at the end of 10 weeks on MS nutrient medium having Kinetin + NAA are shown in Table 1. Visible calli were induced on explants subjected to all treatments except control. The least callus formation was induced on the medium having 4.65 µM Kinetin + 2.685 µM NAA. Similarly, Vaziri (2014) found that all leaf scale explants formed callus in all culture treatments. Considering the bulb diameters, the maximum number of 4.08 bulbs with 0.31 cm diameter were noted on 4.65 µM- 5.37µM NAA having medium (Figure 1.a) and 3 bulbs with 9.30 µM Kinetin + 2.685, 5.37 and 8.74 µM NAA. There was no statistical difference in terms of average bulb diameter.

Table 1. Regeneration on primary bulb explants induced on bulb scales post 10 weeks on MS medium having Kinetin + NAA

Plant Growth Hormone (µM)		Number of 2ndry bulblets per primary bulb	Bulb diameter (cm)
Kinetin	NAA		
4.65	2.685	2.33ab	0.27
4.65	5.370	4.08a	0.31
4.65	8.740	1.17b	0.26
9.30	2.685	3.00ab	0.28
9.30	5.370	3.00ab	0.27
9.30	8.740	3.00ab	0.35
18.60	2.685	1.00b	0.34
18.60	5.370	2.67ab	0.30
18.60	8.740	1.42ab	0.37
MS medium (control)		1.25b	0.36

The means of all values shown in single columns expressed with different letters point out that they are statistically different at $p<0.01$ level of significance using the Tukey's b test.

Primary Bulbs as Explants

M. racemosum bulbs with the largest diameter were obtained after 20 weeks (See Table 2, 2nd column). They were sub-cultured (Table 1) to improve their bulb diameter. The initial diameters of primary bulbs varied between 0.23-0.44 cm (Table 2). The culture treatment with the largest primary bulbs (0.76 cm) was MS medium having 18.60 µM Kinetin +5.37 µM NAA.

Secondary bulb formation did not occur in the control group on MS medium having 18.60 µM Kinetin + 8.74 µM NAA. 0.92 secondary bulbs were formed on primary bulbs in the medium having 4.65 µM Kinetin + 5.37 µM NAA, where the best results were obtained in the first regeneration study (Figure 1.b-c). The diameters of the secondary bulbs varied between 0.14-0.31 cm. The number of secondary bulbs per explant varied between 0.42-0.92 per primary bulb used as explant. Callus formation was observed in all treatments except the control and treatments having 9.30 µM Kinetin + 5.37 and 10.74 µM NAA. It was observed that the leaves of all bulbs turned yellow in the medium having 18.60 µM Kinetin +5.37 and 8.74µM NAA (Figure 1.d-e). It was assumed that the high doses of Kinetin and NAA used in the treatment-induced abiotic stress on the chlorophyll contents of the leaf blades ending up with chlorosis.

Table 2. Regeneration of primary bulbs obtained from scale leaves on MS medium having Kinetin + NAA after 20 weeks by subculturing

Plant Growth Hormone (µM)		The initial diameter of primary bulbs (cm)	Final diameter (cm)	The difference in initial and final diameter (cm)	Percentage (%) of 2ndry bulblets	The average diameter of 2ndry bulblets	Number of bulblets per magenta culture box
Kinetin	NAA						
4.65	2.685	0.34	0.58bc	0.31ab	50.00a	0.30	0.92
4.65	5.370	0.38	0.67abc	0.38ab	41.66b	0.31	0.92
4.65	8.740	0.23	0.69abc	0.16b	50.00a	0.31	0.83
9.30	2.685	0.36	0.53c	0.16b	50.00a	0.14	0.42
9.30	5.370	0.39	0.53c	0.14b	25.00c	0.17	0.50
9.30	8.740	0.36	0.67abc	0.31ab	58.00aa	0.30	0.67
18.60	2.685	0.31	0.57bc	0.26ab	25.00c	0.18	0.42
18.60	5.370	0.40	0.76a	0.36ab	58.00aa	0.28	0.92
18.60	8.740	0.44	0.73ab	0.30ab	00.00d	0.00	0.00
MS medium (control)		0.34	0.37d	0.21ab	00.00d	0.00	0.00

The means of all values shown in single columns expressed with different small letters point out that they are statistically different at $p<0.01$ level of significance using the Tukey's b test.

The largest primary bulbs with a diameter of 0.76 cm were induced on MS medium having 18.60 µM Kinetin + 5.37 µM NAA. Similarly, Vaziri et al. (2014) induced 51.7 bulbs per two-scale explants of *M. aucheri* in a medium having 9.30 µM Kinetin + 0.83 µM IBA. The highest number of bulbs per immature embryo (18.3 units) was obtained on MS medium having 2.325 µM Kinetin, 8.88 µM BAP, and 1.225 µM IBA. Ozel et al. (2009) obtained 100 % bulblet regeneration using 9.30µM Kinetin and 2.685 µM NAA on *M. macrocarpum* in MS medium. The bulb diameters increased using subculture. They observed an increase in diameter in the medium having 18.60µM Kinetin -8.74 µM NAA, whereas induction of secondary bulbs was not noted. Uranbey et al. (2010) reported the maximum regeneration on explants of *M. azureum*. They noted 34.5 bulblets per explant on MS medium having 4.65 µM Kinetin on explants with two scales, the highest number of 41 bulblets per explant were noted on explants with four scales on MS medium having 9.30 µM Kinetin. Uranbey (2010b) propagated *M. aucheri* using 2-4 D on bulb scales using Orchimax and Nitsch & Nitsch Media fortified with 9.06µM 2,4-D, 20 mg L^{-1}

mannitol, 20 g L^{-1} sucrose, 2.685 µM NAA, and several doses of BAP, Kinetin, 2-iP and thidiazuron on 2 g L^{-1} gelrite solidified medium. The bulblet induction was noted on both media using BAP, Kinetin, and 2-iP on 2-4 bulb scales. The maximum number of bulblets was noted on the Orchimax medium fortified with 4.65 µM Kinetin and 9.30 µM Kinetin. When the primary bulbs were subcultured as explant, 58% secondary lateral bulb formation was observed on them with an average diameter of 0.28 cm on a medium having 9.30 µM Kinetin + 7.40µM NAA. Similarly, Ozel et al. (2009) noted 100% secondary bulbs formation for each primary bulb grown on a medium having 4.65 µM Kinetin + 2.65 µM NAA in *M. macrocarpum*, but in this case chlorosis in primary bulbs was observed. Similarly, Çetin et al. (2007) determined a high rate of chlorosis on the leaves when they examined the *in vitro* regeneration abilities of the shoot tip culture of *Dianthus caryophyllus* L. in a medium having 2.45 µM IBA and 4.65 µM Kinetin.

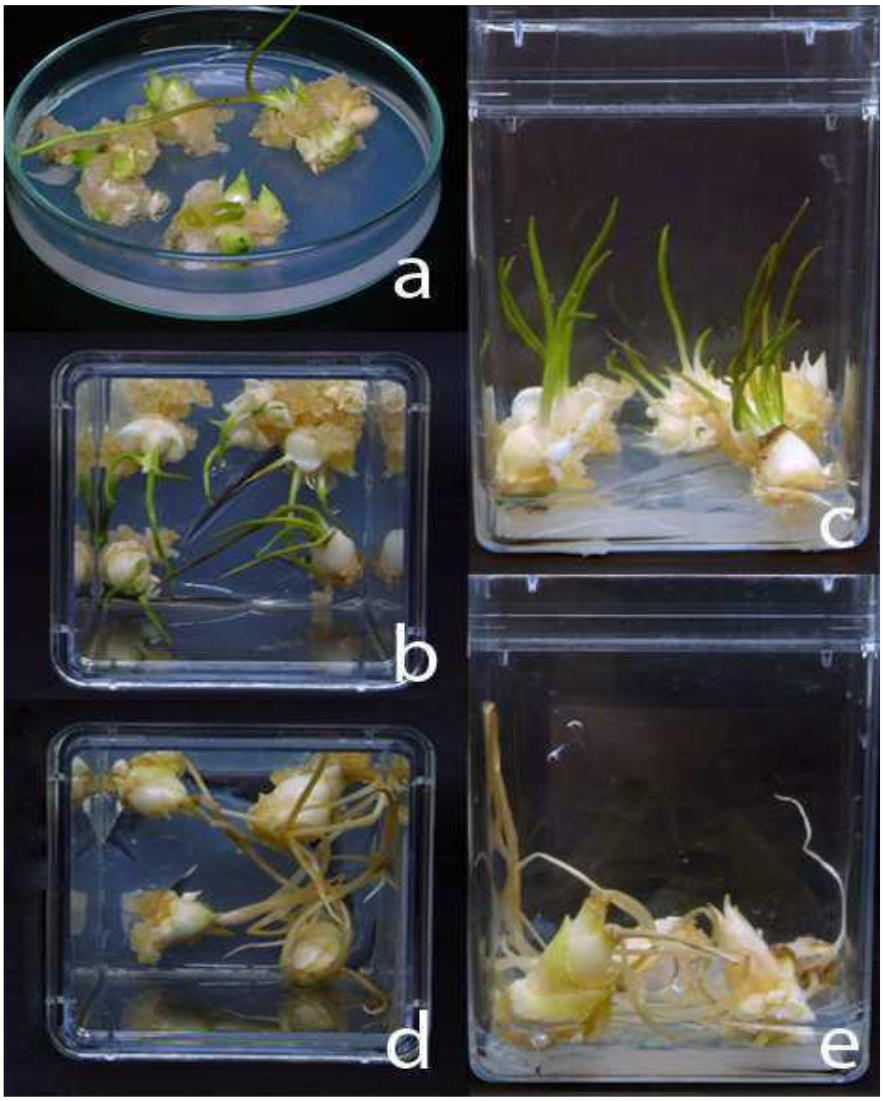

Figure 1. Regeneration of *M. racemosum* in MS medium having Kinetin + NAA (a) bulblet grown in a medium having 4.65µM KIN-5.37 µM NAA after 10 weeks of culture (bc) development of secondary bulblets on primary bulblets in MS medium having 4.65 µM Kinetin 5.37 µM (de) chlorosis on leaf blades using 4 µM KIN- 8.74µM NAA on primary bulblet

Rooting of the Bulbs on a Medium Having 4.90 µM IBA

The bulbs (See Table 2, 3rd column) were rooted on 4.90 µM IBA after six weeks (Table 3). Since the bulbs were taken from several culture treatments, the initial diameters of the bulbs varied between 0.37 and 0.78 cm. The initial diameters of 0.76 and 0.78 cm were determined in bulbs obtained from the MS medium having 18.60 µM Kinetin +5.37 and 8.740 µM NAA. The bulbs having 4.65 µM Kinetin+ 8.74 µM NAA and 18.60 µM Kinetin + 8.74 µM NAA with diameter ranges of 0.90 - 0.97 cm were rooted on 4.90 µM IBA. The greatest increase in diameter was obtained on bulbs taken on a medium having 18.60 µM Kinetin + 2.685 µM NAA. Rooting percentages per explant varied between 25 and 100%. A small number of undeveloped root tips were found in the medium having 18.60 µM Kinetin + 8.74 µM NAA (Figure 2.a-b). The maximum number of roots (3.92) were obtained on the bulbs which were regenerated on 4.65 µM Kinetin + 8.74 µM NAA. The longest roots were noted on this medium after the control group (Figure 2.c-d). Uranbey (2010a) rooted *M. azureum* bulbs on ½ × MS medium fortified with 4.90 µM IBA.

Table 3. Rooting of the bulbs regenerated on several doses of Kinetin + NAA (column 1 and 2) using 4.90 µM IBA

Plant Growth Hormone (µM)		The initial diameter of bulbs	The final diameter of bulbs (cm)	The difference in initial and final diameter of bulbs (cm)
Kinetin	NAA			
4.65	2.685	0.58abc	0.71ab	0.12
4.65	5.370	0.67abc	0.84a	0.18
4.65	8.740	0.70ab	0.97a	0.28
9.30	2.685	0.49abc	0.72ab	0.23
9.30	5.370	0.42bc	0.57ab	0.15
9.30	8.740	0.55abc	0.72ab	0.17
18.60	2.685	0.52abc	0.65ab	0.34
18.60	5.370	0.76a	0.94a	0.19
18.60	8.740	0.78a	0.90a	0.12
MS medium (control)		0.37c	0.38b	0.01
Plant Growth Hormone (µM)		Rooting percentage (%)	Number of roots per bulblet	Average root length (cm)
Kinetin	NAA			
4.65	2.685	58.33c	1.08bc	2.59ab
4.65	5.370	91.67b	2.83ab	1.96ab
4.65	8.740	100.00a	3.92a	4.74ab
9.30	2.685	91.67b	2.08abc	4.45ab
9.30	5.370	25.00e	1.58bc	3.33ab
9.30	8.740	58.33c	1.25bc	2.71ab
18.60	2.685	25.00e	0.33c	4.30ab
18.60	5.370	58.00c	0.75bc	1.15ab
18.60	8.740	33.33de	0.58c	0.04b
MS medium (control)		100.00a	1.00bc	5.18a

The means of all values shown in single columns expressed with by different small letters point out that they are statistically different at $p<0.05$ and 0.01 level of significance using the Tukey's b test

Adaptation of *M. racemosum* bulbs

The rooted bulbs were acclimatized to the external conditions in pots (Figure 2.e). The developments on the number of roots per bulb and their root lengths were examined by removing them from the pots to determine if adaptation affected morphological changes in rooting after eight weeks (Table 4).

Table 4. Morphological developments on roots of bulblets regenerated on several doses of Kinetin + NAA (column 1 and 2) after 8 weeks of rooting and acclimatization

Plant Growth Hormone (µM)		Rooting Percentage (%)	Number of roots per bulblet	Average root length (cm)
Kinetin	NAA			
4.65	2.685	100.00a	2.16ab	8.00b
4.65	5.370	100.00a	2.11ab	4.13d
4.65	8.740	100.00a	2.98a	5.18c
9.30	2.685	100.00a	1.50b	10.92a
9.30	5.370	100.00a	2.15ab	6.46c
9.30	8.740	100.00a	1.67b	3.67d
18.60	2.685	100.00a	1.00c	3.27d
18.60	5.370	100.00a	0.25c	0.40e
18.60	8.740	25.00b	0.25c	0.02e

The means of all values shown in single columns expressed with different small letters point out that they are statistically different at $p<0.05$ and 0.01 level of significance using the Tukey's b test.

The rooting ranged from 25 to 100% and the number of new roots per explant varied between 0.25 and 2.98. The maximum number of roots was obtained on the bulbs regenerated on MS medium having 4.65 µM Kinetin + 5.37 µM NAA. Root lengths varied between 0.02 and 10.92 cm. The longest roots were obtained on the bulbs regenerated on MS medium having 9.30 µM Kinetin + 2.685 µM NAA (Figure 2.f). It was observed that lateral roots emerged from the main roots. Azad and Amin (2012) rooted 2 - 4 cm diameter bulbs of *M. armeniacum* Leichtil. ex Bak on MS medium supplemented with several doses (0.5 - 4.0 µM) of IBA. Similarly, Uzun et al. (2014) rooted *M. muscarimi* bulblet induced on immature zygotic embryos and noted 59 bulblets/explant on MS medium using 4.44µM BAP and 2.685 µM NAA after 365 d. However, they also rooted the bulblets on MS rooting medium and noted increased size after two months. Only 5% of the rooted bulbs were successfully acclimatized to external conditions.

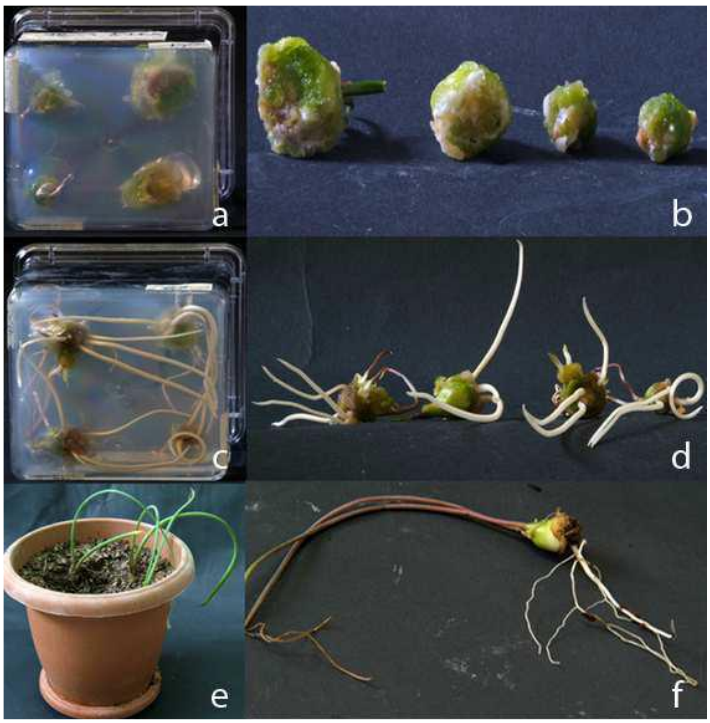

Figure 2. Rooting and adaptation of bulbs induced on *M. racemosum* bulb scales in a medium having 4.90 µM IBA (ab) bulbs taken from a medium having 18.60 µM Kinetin + 8.74 µM NAA and (c, d) rooted using 4.90 µM IBA (e) The acclimatized bulbs in pots (f) developing roots after eight weeks of culture in pots

Leaf bases as explants

Initially, the bulbs were induced under *in vitro* conditions on bulb scales using MS medium having BAP + NAA. Thereafter, the basal parts of the leaf blades were cut into 1 cm long explants and left for regeneration. These induced new bulblets after 4-5 weeks, at the end of 8 weeks. The leaf blade explants were fully consumed, from the regenerated bulbs were both subcultures with transference to several culture media to increase their diameter (Table 5). It was observed that the best medium in terms of the number of bulbs per leaf explant was 17.76 µM BAP + 2.685 µM NAA, and all of the regenerated bulbs were healthy, green, and had 2-3 cm leaves. The largest diameters were obtained from 17.76 µM BAP + 8.74 µM NAA medium. Callus did not form in the medium having 4.44 µM BAP + 2.685 µM NAA and 17.76 µM BAP + 2.685 µM NAA (Figure 3.a). It was observed that the best treatment for bulb induction per explant was the MS medium having 17.76 µM BAP + 2.685 µM NAA (Figure 3.b). Several green bulb initiating tips were observed on the explants. Similarly, Nasırcılar et al. (2011) used leaf explants using picloram+ 2,4-D +NAA, along with doses of BAP for bulblet to regenerate from bulb scales and leaves of *M. mirum*. However, they did not observe induction of any bulb on leaf and bulb scale explants. Azad and Amin (2012) developed an *in vitro* propagation system for *M. armeniacum*. A range of 17.76 µM BAP or 8.74 µM NAA concentration was investigated for bulblet regeneration on the explants. Only leaf-sheath explants of *in vitro* grown bulblets induced direct adventitious bulblets. The best (100%) bulblet regeneration was noted on 17.6 µM BAP + 8.74 µM NAA. Wang et al. (2013) developed a system of plant regeneration on leaf explants of *M. armeniacum* via somatic embryogenesis. They used 2.265 µM 2,4-D and 0.1 µM TDZ having MS basal medium with a high frequency of indirect somatic embryo production, while MS basal medium supplemented with 0.1 µM BA and 0.454 µM TDZ exhibited a high frequency of direct somatic embryogenesis on cut leaf explants. Mori and Nakano (2004) noted that flower bud-derived explants of *M. armeniacum* had the highest tendency to induce callus and somatic embryos in comparison to calli induced on leaves. They noted that *M. armeniacum* cv. Blue Spike induced leaf-derived calli (46.7 %) and flower-bud-derived calli also induced somatic embryos (63.3 %).

Table 5. Regenerating bulblets on leaf blade induced bulbs obtained under *in vitro* conditions

Plant Growth Hormone (µM)		Average vitality percentage per magenta box	Percentage of bulblet induction	Number of bulblets per explant	The average diameter of induced bulblets	Callus induction percentage per magenta box
BAP	NAA					
4.44	2.685	73.33b	66.67abc	3.06ab	0.22ab	0.00b
4.44	5.370	100.00a	73.33ab	3.07ab	0.18b	100.00a
4.44	8.740	73.33b	40.00cd	3.08ab	0.17b	100.00a
8.88	2.685	100.00a	53.33bcd	3.17ab	0.17b	100.00a
8.88	5.370	93.33ab	73.33ab	3.20ab	0.15b	93.33a
8.88	8.740	80.00b	73.33ab	2.08b	0.15b	93.33a
17.76	2.685	100.00a	93.33a	4.50a	0.21ab	0.00b
17.76	5.370	100.00a	53.33abc	2.33ab	0.23ab	73.33ab
17.76	8.740	100.00a	26.67de	2.83ab	0.32a	100.00a
MS medium (control)		0.00c	0.00e	0.00c	0.00c	0.00b

The means of all values shown in single columns expressed with several small letters point out that they are statistically different at $p<0.01$ level of significance using the Tukey's b test.

They emphasized that leaves are better explants compared to flower buds as they are available in large numbers throughout the year. Suzuki and Nakano (2001) induce

Rooting bulbs obtained from leaf blades at 4.90 µM IBA

The bulbs with the largest diameter were selected (See Table 5 4th column) and regeneration on the root, bulb scale, flower stalk, and leaf explants of *M. armeniacum* and noted that the leaf explants induced the highest percentage of calli.

rooted on MS medium having 4.90 µM IBA. Bulbs were counted after eight weeks (Table 6).

Table 6. Rooting of the bulbs regenerated on several doses of BAP +NAA (column 1 and 2) using 4.90 µM IBA

Plant Growth Hormone (µM)		The initial diameter of the bulbs	The final diameter of the bulbs	The difference in the diameter of the bulbs
BAP	NAA			
4.44	2.685	0.27b	0.59	0.32
4.44	5.370	0.24b	0.49	0.25
4.44	8.740	0.40a	0.62	0.22
8.88	2.685	0.21b	0.43	0.22
8.88	5.370	0.30ab	0.67	0.37
8.88	8.740	0.24b	0.47	0.23
17.76	2.685	0.29ab	0.64	0.35
17.76	5.370	0.32ab	0.56	0.24
17.76	8.740	0.33ab	0.57	0.24
Plant Growth Hormone (µM)		Rooting percentage	Number of roots per bulb	Average root length (cm)
BAP	NAA			
4.44	2.685	0.00c	0.00b	0.00
4.44	5.370	100.00a	1.65a	1.27
4.44	8.740	0.00c	0.00b	0.00
8.88	2.685	36.67 b	0.37b	0.75
8.88	5.370	3.33c	0.03b	0.17
8.88	8.740	0.00c	0.00b	0.83
17.76	2.685	10.00a	0.10b	0.73
17.76	5.370	10.00a	0.10b	1.08
17.76	8.740	5.00c	0.05b	0.25

The means of all values shown in single columns expressed with by different small letters point out that they are statistically different at $p<0.01$ level of significance using the Tukey's b test

The largest diameters were noted on the medium having 4.44 µM BAP + 8.74 µM NAA. The final diameters ranged between 0.43 and 0.67 cm and the bulb difference between the initial and final diameters varied from 0.22 to 0.37 cm. The maximum number of 1.65 bulblets per explant was noted with the length of 1.27 cm on MS nutrient medium having 4.44 µM BAP + 5.37 µM NAA (Figure 3.c). At the end of 8 weeks, the bulbs were adapted to the soil with 100% viability (Figure 3.d). These were rooted on 4.14 µM IBA. The results are similar to Faruq et al. (2018) who showed the maximum percentage of rooting on half-strength MS medium having 8.28 µM IBA. However, all the auxins >2.0 µM concentration showed reduced root formation. A potential reason could be the induction of callus at the base of shoots or malformation of roots. All *in vitro* regenerated bulblets of *M. armeniacum* were successfully acclimatized under *ex vitro* conditions with a 60% survival rate using peat.

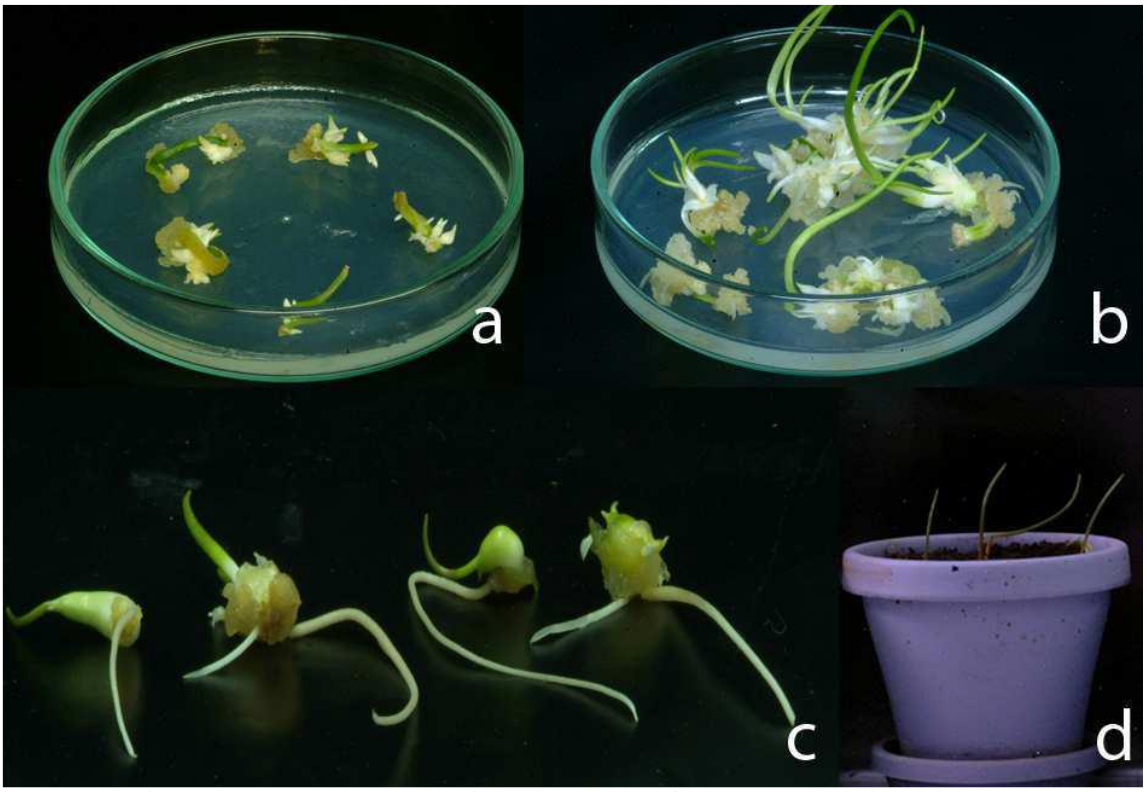

Figure 3. Rooting of *M. racemosum* bulbs induced on leaf bases (a) Beginning of bulb induction on leaf bases in MS medium having 17.76 µM BAP+ 2.685 µM NAA (b) induction of bulbs after 10 weeks of culture on leaf bases using the same medium (c) rooting of bulblets induced on MS medium having 4.44 µM BAP-5.37 µM NAA using 4.90 µM IBA (d) acclimatized plants in pots

CONCLUSION

The current study provides important information on commercial clonal propagation of *M. racemosum*. The importance of explants (bulb scales, primary bulbs, and leaf bases), Kinetin + NAA and BAP + NAA for regeneration and IBA in rooting is proved explicitly. The results provide visible information on the role of *M. racemosum* explaňt types, their regeneration potential and meet the targets of the study.

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